PATENT COOPERATION TREATY

PCT

REC'D 16 MAR 2005
WIPO PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference BET 03P0869	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/12398	International filing date *(day/month/year)* 26.09.2003	Priority date *(day/month/year)* 27.09.2002
International Patent Classification (IPC) or both national classification and IPC C12Q1/70		
Applicant INSTITUT NATIONAL DE LA SANTE ET ... et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
16.02.2004	17.03.2005
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Botz, J Telephone No. +31 70 340-4513

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/12398

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-34 as originally filed

Claims, Numbers

1-36 as originally filed

Drawings, Sheets

1/6-6/6 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 1-34 (partially), 35, 36 (completely)

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 1-34 (partially), 35, 36 (completely)

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees, the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☒ complied with.

☐ not complied with for the following reasons:

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☐ all parts.

☒ the parts relating to claims Nos. 1-34 (partially) .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1 - 34 (partially)
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1 - 34 (partially)
Industrial applicability (IA)	Yes:	Claims	1 - 34 (partially)
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item III

Due to a non-establishment of the search-report for claims 1 - 34 (partially), 35 and 36 (both completely) no opinion will be provided on these claims with respect to industrial applicability, novelty and inventive step.

Re Item IV

3. Lack of unity of invention

3.1 In contrast to the ISA the IPEA considers the requirement for unity of invention complied with in the underlying application (**Rule 13.1 PCT**).

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:

D1: SCHORIES MARCUS ET AL: 'Isolation, characterization and biological significance of hepatitis B virus mutants from serum of a patient with immunologically negative HBV infection.' JOURNAL OF HEPATOLOGY, vol. 33, no. 5, November 2000 (2000-11), pages 799-811, XP002246280 ISSN: 0168-8278

D2: ULRICH P P ET AL: 'A PRECORE-DEFECTIVE MUTANT OF HEPATITIS B VIRUS ASSOCIATED WITH E ANTIGEN-NEGATIVE CHRONIC LIVER DISEASE' JOURNAL OF MEDICAL VIROLOGY, vol. 32, no. 2, 1990, pages 109-118, XP008019147 ISSN: 0146-6615

D3: GUNTHER STEPHAN ET AL: 'Amplification of full-length hepatitis B virus genomes from samples from patients with low levels of viremia: Frequency and functional consequences of PCR-introduced mutations.' JOURNAL OF CLINICAL MICROBIOLOGY, vol. 36, no. 2, February 1998 (1998-02), pages 531-538, XP002246281 ISSN: 0095-1137

D4: DELANEY W E 4TH ET AL: 'Use of the hepatitis B virus recombinant baculovirus-HepG2 system to study the effects of (-)-beta-2',3'-dideoxy-3'-thiacytidine on replication of hepatitis B virus and accumulation of covalently closed circular DNA.'ANTIMICROBIAL AGENTS AND CHEMOTHERAPY. UNITED STATES AUG 1999, vol. 43, no. 8, August 1999 (1999-08), pages 2017-2026, XP002246282 ISSN: 0066-4804

D5: DATABASE EBI [Online] 6 October 1999 (1999-10-06) FRANK, B.L., ET AL.: 'Sequence 5 from patent US 5856459' Database accession no. AR027807 XP002246286 & US 5856459 A (HYBRIDON, INC.) 5 January 1999 (1999-01-05)

D6: DATABASE GENBANK [Online] 23 March 2001 (2001-03-23) SUGAUCHI, F. ET AL.: 'Hepatitis B virus DNA, complete genome, serotype:ayw' retrieved from GI:13365548, accession no. NCBI Database accession no. AB048704 XP002246287 cited in the application

2. **Novelty (Article 33 (2) EPC)**

2.1 The subject-matter of **claims 1 - 34** (partially) is considered to be new in the sense of **Article 33(2) PCT**, and therefore meets the criteria of **Article 33(1) PCT**.

3. **Inventive Step (Article 33(3) PCT)**

3.1 The present application does not meet the criteria of **Article 33(1) PCT**, because the subject-matter of **claims 1 - 34** (partially) does not involve an inventive step in the sense of **Article 33(3) PCT**. The reasons are as follows:

3.2 **D3** is considered to represent the closest prior art. **D3** represents a method for measuring the replication capacity of HBV. Full length HBV genomes from patient samples were isolated, PCR amplified and subsequently tested in transfection studies using hepatoma cell lines. The PCR-amplified and purified HBV genome is thereby tested by direct transfection, c.f. page 531, left column, second paragraph and paragraph on "Preparation of PCR products for transfection" in the section Materials and Methods as well

as the paragraph on "Transfection of HBV DNA by calcium phosphate precipitation". Unlike in the present application, the genome is amplified not by generation of 2 overlapping fragments but by a long range PCR reaction, resulting in a full length genome PCR product. The replication performance is subsequently measured by purifying the HBV genomes from the HuH7 and analyzing said genomes in a southern blot analysis. In the paragraph "Functional analysis of amplified HBV genomes without cloning: optimization and influence of PCR on the HBV phenotype" on page 534 it becomes clear, that the replicative capacity of the transfected, full-length HBV-genomes is measured, c.f. figure 4A. It is also described that the potential of this system for the analysis of the sensitivity of clinical HBV isolates to antiviral drugs was evaluated. Therefore, as described in said paragraph, the IFN-α responsiveness was determined. In summary, a two-step PCR method was established for amplification of HBV genomes from patient samples yielding sufficient product for testing the replicative capacity of said strains in transfection assays in the presence or absence of antiviral drugs to be tested, c.f. page 534.

3.3 The subject-matter of **claims 1 - 30** (partially) **differs** from said closest prior art in that the HBV genome in the underlying application is transfected into the host cells by means of cloning said genome into a vector under the control of a heterologous promoter.

3.4 The **problem to be solved** by the present invention may therefore be regarded as providing *an alternative system* with which to study and measure the replicative capacity of HBV genomes, present in biological samples.

3.5 The **solution** proposed with **claims 1 - 30** (partially) in the underlying application cannot however be considered as involving an inventive step (**Article 33(3) PCT**) for the following reasons:

3.6 The applicant on page 3, second paragraph, refers to **D3** in stating, that this method would be "highly inefficient" and represent therefore "a major limitation of the analysis of all HBV mutants". Furthermore the applicant states, that the "low level of HBV DNA [in **D3**] synthesis hampers the analysis of viral replication and drug susceptibility testing". The IPEA on the other hand has the impression that **D3** provides the advantage of avoiding an intermediate cloning step by directly transfecting the amplified HBV-genome into the host cells. The argument that the method of **D3** would result in only a "low level of HBV DNA" is not supported when studying figure 4. It is furthermore not supported when taking into consideration the example provided on page 31. In this respect **D3** states in the left column of page 534, that "HBV genome populations amplified by two rounds of PCR from a small number of molecules of ... HBV DNA from the serum of a patient worked well in

transfection experiments". The applicant on the other hand provides no comparative data supporting the view that the transfection of cloned HBV-genomes under the control of a heterologous promoter show a *significantly* better performance than what is achievable with the technology of D3. It deserves furthermore mentioning that for the method of the underlying application a heterologous promoter seems to be dispensable: on page 19, last paragraph, the technique of the underlying application is described comprising an homologous, HBV-derived promoter. Therefore the applicant's statement is not supported and consequently, the Examining Division does not share the applicant's view, that the analysis of viral replication and drug susceptibility testing is "hampered" by the technology of D3.

3.7 The method of measuring the replication capacity of HBV whereby the amplified HBV genome isolated from a biological sample is cloned into a vector-construct prior to transfection was known to the skilled in the art, c.f. D3, page 531, left column, second paragraph. An intermediate cloning step therefore constitutes merely one of two straightforward possibilities from which the skilled person would select, in accordance with circumstances, without the exercise of inventive skill, in order to solve the problem posed.

3.8 The genome of the Hepatitis B Virus is known from the data base deposit of prior art D6: in principle, the design of primers from a published and therefore known sequence is subject to routine practitioning. In the present case it is stressed by the applicant, that the primers are designed so as to hybridize to the various HBV genotypes, c.f. page 7, second paragraph of the description. On page 9, it is revealed that the primers disclosed in the application have been designed after multiple sequence alignments. The Examining Division however considers it straightforward, that in order to determine conserved genomic regions, the skilled in the art would undertake multiple sequence alignments to identify said regions. Therefore the sequence identity numbers 1 and 5 listed in **claims 31 - 34** appear to be the result of routine practitioning, in accordance with circumstances, without the exercise of inventive skill, in order to solve the problem posed: the data base deposit of prior art D5 (sequence 5) shows a complete overlap with sequence identity number 1 on a stretch of 19 basepairs. Within the prior art said data base deposit is used in antisense-techniques and carries the name HBV47. It stretches from nucleotide positions 1816 - 1835 of the HBV-genome according to the data base entry. The difference in the 5' part of sequence identity number 1 is due to an inserted NOT1-restriction site, c.f. figures 2 and 3. Furthermore, sequence identity number 1 is encompassed in the data base deposit of prior art D6, representing the complete genome of the Hepatitis B virus, said data base deposit being cited by the applicant in the description as data base entry from which the primers of the underlying invention were derived from.

3.9 The use of the baculovirus expression system, as outlined in **claim 27**, in order to measure the replication competence of Hepatitis B virus is also not inventive but known from the state of the art. Prior art **D4** describes a system, called "HBV-baculovirus HepG2-system", said system being used to evaluate the utility as a tool for antiviral research using 3TC, an established inhibitor of HBV replication.

Jürgen W. Botz